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DAUERCYSTFORMATION OF *TRICHOMONAS* *INTESTINALIS* *

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Ucke (1908), Bohne and Prowazek (1908), and Bensen (1910), have described encystment of *Trichomonas intestinalis*. Bensen (1910) has also described an encystment for *Trichomonas vaginalis* differing from that of *Trichomonas intestinalis*, and Dobell (1908) reports a dauercyst of *Trichomonas batrachorum*. Alexeieff (1911) disputes the nature of the so-described cyst of *Trichomonas intestinalis*, asserting that it is in reality an ascomyces, a vegetable organism akin to the yeasts, and proposes for it the name *Blastocystis enterocola*. Wenyon (1905) and others uphold Alexeieff's contention, Wenyon calling the organism *Blastocystis hominis*.

The question of encystment of *Trichomonas intestinalis* is an interesting one to me because of the questioned nature of the cyst which has been previously described and because of the prevalence of the parasite in this community. For several years I have been observing *Trichomonas* as a parasite of several locations in the human body and in certain lower animals, and have frequently encountered a form which has proven to be a distinct cyst and not to be confused with that previously described as an encysted *Tr. intestinalis* by Ucke, Bohne and Prowazek, and Bensen, and as *Blastocystis enterocola* by Alexeieff.

At the present I have under observation a man who furnishes this cyst in large numbers and with distinct characteristics. This man is a negro who is in the hospital with chronic endocarditis and who gives no history of dysentery.

In the stool no other protozoon has been found, no cell corresponding to the previously described *Tr. intestinalis* cyst and none of the *Blastocystis* of Alexeieff. There are many active *Trichomonas* conforming to the typical organism, averaging about 8 by 12 micra in size, of elongated pear shape, with constant undulating membrane, three flagella, the stiff spine projecting posteriorly, vacuolated cytoplasm with numerous ingested bacteria, and with nucleus indistinct but showing well in stained preparations. On exposure the active form soon becomes inactive, stationary, and does not develop the irregular ameboid, non-flagellated, undulating form. It is one of the most fixed types I have observed.

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The encysted form is almost as numerous as the active and commonly exhibits a tendency to occur in pairs. It is about three-fourths the size of the active, of a typical pear shape, and has a transparent shell of uniform thickness. The enclosed parasite has a regular ovoid contour and a finely granular grayish appearance. On one side nearer to the small end the nucleus is visible as a refractive granule, and on the other the undulating membrane is seen as a refractive wavy line extending from end to end.

More definite observations of this cyst and its different structures may be made from stained specimens. The technic of staining which I have used is as follows: A thin spread of the feces while still wet is fixed in warm saline alcoholic corrosive sublimate for ten minutes, placed in absolute alcohol ten minutes, iodine solution ten minutes, alcohol ten minutes, washed in distilled water, mordanted in 4% aqueous ferric ammonia alum overnight, stained in 1% alcoholic hematoxylin one day, decolorized in 2% aqueous ferric ammonia alum, counterstained in alcoholic eosin and cleared in carbol-xylol.

In such a preparation the encysted form is more deeply stained than the active. It is about 6 by 8 micra in size and of perfectly symmetrical pear shape. The anterior end projects on a shoulder slightly beyond the general line; the wall is uniformly distinct; and the space between the cyst wall and the body of the parasite is usually distinct and clear, it being usually broader at the anterior end.

The body of the enclosed parasite is of symmetrical, ovoid shape, slightly pointed anteriorly, of dull brick-red color, finely granular and contains no vacuoles or food particles. A fine dark line beginning as a granule in the anterior end runs directly backward to the posterior end. This I take to be the stiffening rib of the undulating membrane because of its close association in origin and termination with that organ. The undulating membrane is distinct as a darker line beginning in close connection with this rib and extending backward along one side of the body in a wavy course to the posterior end, where it curves around the extremity of the parasite and comes to end near this end of the rib. The nucleus is comparatively large, of ovoid form, but lies farther back than in the active parasite and on the side opposite to the undulating membrane. Its usual position is in the posterior part of the anterior half, and between the midline and the body wall. It has a distinct dark-stained rim and a large chromatin mass. This chromatin usually occurs as an irregular black body almost filling the nucleus, but is in some broken into smaller granules and in others distributed around the inner edge of the nuclear rim. In addition to the undulating membrane the cyst usually shows two or three more delicate lines arising in close association with that organ and passing backward over the body for about two thirds of its length. These are

probably flagella. They stain poorly and are not constantly seen, especially in the more faintly stained specimens. The characteristics of the fully formed cyst may be seen in Figure A3 which is an off-hand drawing.

In addition to this fully developed cyst which predominates, there are young cysts and forms of apparent pre-encystment. There is a form which is smaller than the active parasite, shorter and more blunt, with cytoplasm somewhat reticulated, but showing no vacuoles and no bacteria or other ingested materials, and with nucleus more distinct and with larger amounts of chromatin. This I take to be a pre-encysted stage. Figure A1 appears to be a young cyst before the wall has reached full formation. Its shape is typical; the body wall is thick; and the internal organs appear as in the fully encysted. A further stage appears to be the form in which there is a space between the shell and parasite only at the anterior end, these parts being in immediate contact around the remainder of the body, see A2.

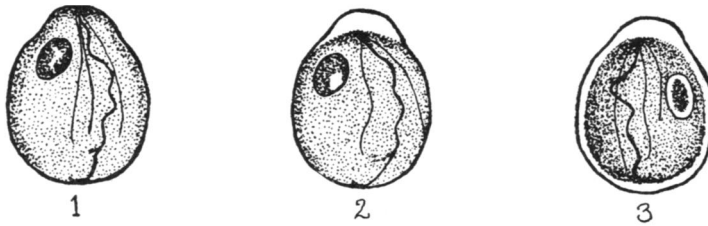


Fig. A1, 2, and 3.—Drawings of different stages of dauercysts of *Trichomonas intestinalis* as seen in specimens stained in hematoxylin.

Further development suggestive of multiplication I have not seen in these cysts; and the preservation of the undulating membrane and flagella, together with the single nucleus, indicate that the process is not for reproduction but simply for resistance.

DISCUSSION

According to these observations the formation of a resistance cyst plays a part in the life of *Trichomonas intestinalis* as occurs with other intestinal protozoa, and I believe is the stage in which the parasite may be transmitted. That infection by the contracted but non-encysted parasite occurs I am not prepared to dispute, and it seems probable to me that the contracted form may be a pre-encystment stage. Reasoning by analogy leads one to believe that infection through the stomach by means of the non-encysted is not probable, and certain observations which I have made of the purely active *Trichomonas* from the vagina also rule against such a manner of infection.

I have previously reported (1915) a case of vaginal and mouth infection by *Trichomonas* which did not infect the intestine, this being determined by repeated examination after purging. I have since had a similar case of trichomoniasis of the vagina and mouth; and again by repeated antemortem and also postmortem examinations failed to find the intestine infected. In these cases the parasites seemed identical in the two situations, and there was never any but the pure fixed type of active form. There were enormous numbers in the mouth for

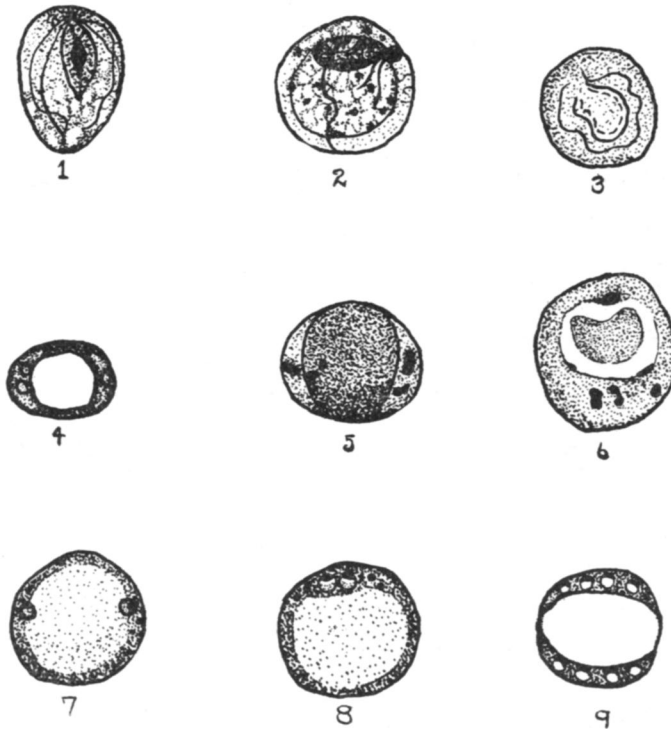


Fig. B1, Dauercyst of *Tr. batrachorum* by Dobell; 2, Encysted *Tr. vaginalis* by Bensen; 3, Cyst of *Tr. intestinalis* by Wenyon; 4, Encysted *Tr. intestinalis* by Ucke; 5, Encysted *Tr. intestinalis* by Bohne and Prowazek; 6, Encysted *Tr. intestinalis* by Bensen; 7 and 8, *Blastocystis enterocola* by Alexeieff; 9, *Blastocystis hominis* by Wenyon.

considerable periods of time; and if the active *Trichomonas* is capable of transmission through the stomach to the intestine in man, the swallowing of these organisms should have produced an intestinal infection in these women.

It is not my purpose at this time to enter into a discussion as to the nature of the previously described encysted *Tr. intestinalis* which has been called *Blastocystis* by Alexeieff; but from extensive observa-

tion of both organisms, both associated and occurring separately, I am in accord with the view that it is not a *Trichomonas* cyst.

In order that the lack of resemblance of this cell to the cyst here described may be seen I have included copies of figures by Ucke, Bohne and Prowazek, Bensen, Alexeieff, and Wenyon. Figures B5 and 6 are from a hematoxylin-stained specimen. Hence the main difference in appearance to Figures B4, 7, 8, and 9, which are representations of the unstained cell, since the internal part of the cell, which is often transparent in the fresh specimen, stains rather deeply and the nuclei are more distinct in the stained. Figures B4, 5, and 6 are illustrations of the so-called *Trichomonas intestinalis* cysts of Ucke, Bohne and Prowazek, and Bensen. Figures B7, 8, and 9 are illustrations of *Blastocystis* by Alexeieff and Wenyon. There is seen to be no point of similarity between the *Trichomonas* cyst of Ucke, Bohne and Prowazek or Bensen and that here described and pictured in Figure A; whereas, barring differences in preparation and in stage of the organism, the *Blastocystis* of Alexeieff and Wenyon and the *Trichomonas* cyst of Ucke, Bohne and Prowazek and of Bensen are apparently one and the same organism.

There is however some resemblance between this cyst and Bensen's encysted *Trichomonas vaginalis* (Figure B2) and Dobell's cyst of *Trichomonas batrachorum* (Figure B1), which I have taken the liberty of copying for comparison. In the last two the flagella are preserved until the cyst is formed, but lost afterwards; while the typical shape of the *Trichomonas intestinalis* cyst is not observed, and the undulating membrane and axostyle are not seen. The nucleus is also different, in that in *Tr. intestinalis* it is more rounded and placed more posteriorly, while in both *Tr. batrachorum* and *Tr. vaginalis* it is forward, larger and spindle shaped. Then in the case of *Tr. vaginalis* Bensen has described and illustrated further development of the cyst for multiplication, while further development of the cyst here described has not been seen.

CONCLUSION

Accordingly, therefore, the formation of a resistance cyst does play a part in the life of *Trichomonas intestinalis*, and this cyst bears no relationship to the cell which has been previously described as an encysted *Tr. intestinalis* by Ucke and others, and as *Blastocystis enterocola* by Alexeieff, and differs essentially from Dobell's dauer-cyst of *Trichomonas batrachorum* and Bensen's encysted *Tr. vaginalis*.

SUMMARY

Encystment of *Trichomonas intestinalis* has been investigated for many years. That which has been previously described has not stood

the test of investigation. The morphology of the cyst here described identifies it with the parasite from which it arises. It is not to be confused with any cell occurring in the intestine and feces. Whether this cyst releases more than one organism or whether *Trichomonas intestinalis* has a multiplication cyst remains an unanswered question.

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